Frontiers in Pharmacology, 2018, vol.9, NFEB

Triple-gene therapy for stroke: A proof-of-concept in vivo study in rats

Kazan Federal University, 420008, Kremlevskaya 18, Kazan, Russia

Abstract

© 2018 Sokolov, Bashirov, Markosyan, Povysheva, Fadeev, Izmailov, Kuztetsov, Safiullov, Shmarov, Naroditskyi, Palotás and Islamov. Natural brain repair after stroke is extremely limited, and current therapeutic options are even more scarce with no clinical break-through in sight. Despite restricted regeneration in the central nervous system, we have previously proved that human umbilical cord blood mono-nuclear cells (UCB-MC) transduced with adenoviral vectors carrying genes encoding vascular endothelial growth factor (VEGF), glial cell-derived neurotrophic factor (GDNF), and neural cell adhesion molecule (NCAM) successfully rescued neurons in amyotrophic lateral sclerosis and spinal cord injury. This proof-of-principle project was aimed at evaluating the beneficial effects of the same triple-gene approach in stroke. Rats subjected to distal occlusion of the middle cerebral artery were treated intrathecally with a combination of these genes either directly or using our cell-based (UCB-MC) approach. Various techniques and markers were employed to evaluate brain injury and subsequent recovery after treatment. Brain repair was most prominent when therapeutic genes were delivered via adenoviral vector- or UCB-MC-mediated approach. Remodeling of brain cortex in the stroke area was confirmed by reduction of infarct volume and attenuated neural cell death, depletion of astrocytes and microglial cells, and increase in the number of oligodendroglial cells and synaptic proteins expression. These results imply that intrathecal injection of genetically engineered UCB-MC over-expressing therapeutic molecules (VEGF, GDNF, and NCAM) following cerebral blood vessel occlusion might represent a novel avenue for future research into treating stroke.

http://dx.doi.org/10.3389/fphar.2018.00111

Keywords

Adenoviral vector, GDNF, Gene therapy, Human umbilical cord blood mono-nuclear cell, Middle cerebral artery, NCAM, Stroke, VEGF

References

[1] Abdellatif, A. A., Pelt, J. L., Benton, R. L., Howard, R. M., Tsoulfas, P., Ping, P., et al. (2006). Gene delivery to the spinal cord: comparison between lentiviral, adenoviral, and retroviral vector delivery systems. J. Neurosci. Res. 84, 553-567. doi: 10.1002/jnr.20968

[2] Aiuti, A., Roncarolo, M. G., and Naldini, L. (2017). Gene therapy for ADA-SCID, the first marketing approval of an ex vivo gene therapy in Europe: paving the road for the next generation of advanced therapy medicinal products. EMBO Mol. Med. 9, 737-740. doi: 10.15252/emmm.201707573

[3] Ballen, K. K., Gluckman, E., and Broxmeyer, H. E. (2013). Umbilical cord blood transplantation: the first 25 years and beyond. Blood 122, 491-498. doi: 10.1182/blood-2013-02-453175

[4] Boltze, J., Schmidt, U. R., Reich, D. M., Kranz, A., Reymann, K. G., Strassburger, M., et al. (2012). Determination of the therapeutic time window for human umbilical cord blood mononuclear cell transplantation following experimental stroke in rats. Cell Transplant. 21, 1199-1211. doi: 10.3727/096368911X589609

[5] Cartier, N., Hacein-Bey-Abina, S., Bartholomae, C. C., Veres, G., Schmidt, M., Kutschera, I., et al. (2009). Hematopoietic stem cell gene therapy with a lentiviral vector in X-linked adrenoleukodystrophy. Science 326, 818-823. doi: 10.1126/science.1171242

[6] Cavazzana-Calvo, M., Hacein-Bey, S., de Saint Basile, G., Gross, F., Yvon, E., Nusbaum, P., et al. (2000). Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease. Science 288, 669-672. doi: 10.1126/science.288.5466.669

[7] Chen, B., Zhang, F., Li, Q., Gong, A., and Lan, Q. (2015). Protective effect of ad-vegf-bone mesenchymal stem cells on cerebral infarction. Turk. Neurosurg. 26, 8-15. doi: 10.5137/1019-5149.JTN.11488-14.3

[8] Chen, H. K., Hung, H. F., Shyu, K. G., Wang, B. W., Sheu, J. R., Liang, Y. J., et al. (2005). Combined cord blood stem cells and gene therapy enhances angiogenesis and improves cardiac performance in mouse after acute myocardial infarction. Eur. J. Clin. Invest. 35, 677-686. doi: 10.1111/j.1365-2362.2005.01565.x

[9] Chen, Y., Zhu, H., Liao, J., Yi, Y., Wang, G., Tong, L., et al. (2014). Regulation of naotai recipe on the expression of HIF-la/VEGF signaling pathway in cerebral ischemia/reperfusion rats. Zhongguo Zhong Xi Yi Jie He Za Zhi 34, 1225-1230

[10] Cheng, H., Wu, J.-P., and Tzeng, S.-F. (2002). Neuroprotection of glial cell line-derived neurotrophic factor in damaged spinal cords following contusive injury. J. Neurosci. Res. 69, 397-405. doi: 10.1002/jnr.10303

[11] Chernykh, E. R., Shevela, E. Y., Starostina, N. M., Morozov, S. A., Davydova, M. N., Menyaeva, E. V., et al. (2016). Safety and therapeutic potential of M2 macrophages in stroke treatment. Cell Transplant. 25, 1461-1471. doi: 10.3727/096368915X690279

[12] Colombo, F., and Meldolesi, J. (2015). L1-CAM and N-CAM: from adhesion proteins to pharmacological targets. Trends Pharmacol. Sci. 36, 769-781. doi: 10.1016/j.tips.2015.08.004

[13] Craig, A. J., and Housley, G. D. (2016). Evaluation of gene therapy as an intervention strategy to treat brain injury from stroke. Front. Mol. Neurosci. 9:34. doi: 10.3389/fnmol.2016.00034

[14] Drury-Stewart, D., Song, M., Mohamad, O., Guo, Y., Gu, X., Chen, D., et al. (2013). Highly efficient differentiation of neural precursors from human embryonic stem cells and benefits of transplantation after ischemic stroke in mice. Stem Cell Res. Ther. 4:93. doi: 10.1186/scrt292

[15] Ferrara, N., Houck, K., Jakeman, L., and Leung, D. W. (1992). Molecular and biological properties of the vascular endothelial growth factor family of proteins. Endocr. Rev. 13, 18-32. doi: 10.1210/edrv-13-1-18

[16] Hacein-Bey Abina, S., Gaspar, H. B., Blondeau, J., Caccavelli, L., Charrier, S., Buckland, K., et al. (2015). Outcomes following gene therapy in patients with severe Wiskott-Aldrich syndrome. JAMA 313, 1550-1563. doi: 10.1001/jama.2015.3253

[17] He, B., Yao, Q., Liang, Z., Lin, J., Xie, Y., Li, S., et al. (2016). The dose of intravenously transplanted bone marrow stromal cells determines the therapeutic effect on vascular remodeling in a rat model of ischemic stroke. Cell Transplant. 25, 2173-2185. doi: 10.3727/096368916X692627

[18] Iannotti, C., Li, H., Yan, P., Lu, X., Wirthlin, L., and Xu, X. M. (2003). Glial cell line-derived neurotrophic factor-enriched bridging transplants promote propriospinal axonal regeneration and enhance myelination after spinal cord injury. Exp. Neurol. 183, 379-393. doi: 10.1016/S0014-4886(03)00188-2

[19] Ikeda, Y., Fukuda, N., Wada, M., Matsumoto, T., Satomi, A., Yokoyama, S.-I., et al. (2004). Development of angiogenic cell and gene therapy by transplantation of umbilical cord blood with vascular endothelial growth factor gene. Hypertens. Res. 27, 119-128. doi: 10.1291/hypres.27.119

[20] Islamov, R. R., Chintalgattu, V., Pak, E. S., Katwa, L. C., and Murashov, A. K. (2004). Induction of VEGF and its Flt-1 receptor after sciatic nerve crush injury. Neuroreport 15, 2117-2121. doi: 10.1097/00001756-20040915--00024

[21] Islamov, R. R., Izmailov, A. A., Sokolov, M. E., Fadeev, F. O., Bashirov, F. V., Eremeev, A. A., et al. (2017a). Evaluation of direct and cell-mediated triple-gene therapy in spinal cord injury in rats. Brain Res. Bull. 132, 44-52. doi: 10.1016/j.brainresbull.2017.05.005

[22] Islamov, R. R., Rizvanov, A. A., Fedotova, V. Y., Izmailov, A. A., Safiullov, Z. Z., Garanina, E. E., et al. (2016). Tandem delivery of multiple therapeutic genes using umbilical cord blood cells improves symptomatic outcomes in ALS. Mol. Neurobiol. 54, 4756-4763. doi: 10.1007/s12035-016-0017-x

[23] Islamov, R. R., Rizvanov, A. A., Mukhamedyarov, M. A., Salafutdinov, I. I., Garanina, E. E., Fedotova, V. Y., et al. (2015). Symptomatic improvement, increased life-span and sustained cell homing in amyotrophic lateral sclerosis after transplantation of human umbilical cord blood cells genetically modified with adeno-viral vectors expressing a neuro-protective factor and a neur. Curr. Gene Ther. 15, 266-276. doi: 10.2174/1566523215666150126122317

[24] Islamov, R. R., Sokolov, M. E., Bashirov, F. V., Fadeev, F. O., Shmarov, M. M., Naroditskiy, B. S., et al. (2017b). A pilot study of cell-mediated gene therapy for spinal cord injury in mini pigs. Neurosci. Lett. 644, 67-75. doi: 10.1016/j.neulet.2017.02.034

[25] Izmailov, A. A., Povysheva, T. V., Bashirov, F. V., Sokolov, M. E., Fadeev, F. O., Garifulin, R. R., et al. (2017). Spinal cord molecular and cellular changes induced by adenoviral vector-and cell-mediated triple gene therapy after severe contusion. Front. Pharmacol. 8:813. doi: 10.3389/fphar.2017.00813

[26] Jeong, H., Yim, H. W., Cho, Y., Kim, Y.-I., Jeong, S.-N., Kim, H., et al. (2014). Efficacy and safety of stem cell therapies for patients with stroke: a systematic review and single arm meta-analysis. Int. J. Stem Cells 7, 63-69. doi: 10.15283/ijsc.2014.7.2.63

[27] Jin, L., Neff, T., and Blau, C. A. (1999). Marrow sensitization to 5-fluorouracil using the ligands for Flt-3 and c-Kit. Exp. Hematol. 27, 520-525. doi: 10.1016/S0301-472X(98)00039-3

[28] Karlupia, N., Manley, N. C., Prasad, K., Schäfer, R., and Steinberg, G. K. (2014). Intraarterial transplantation of human umbilical cord blood mononuclear cells is more efficacious and safer compared with umbilical cord mesenchymal stromal cells in a rodent stroke model. Stem Cell Res. Ther. 5:45. doi: 10.1186/scrt434

[29] Kobayashi, T., Ahlenius, H., Thored, P., Kobayashi, R., Kokaia, Z., and Lindvall, O. (2006). Intracerebral infusion of glial cell line-derived neurotrophic factor promotes striatal neurogenesis after stroke in adult rats. Stroke 37, 2361-2367. doi: 10.1161/01.STR.0000236025.44089.e1

[30] Kranz, A., Wagner, D.-C., Kamprad, M., Scholz, M., Schmidt, U. R., Nitzsche, F., et al. (2010). Transplantation of placenta-derived mesenchymal stromal cells upon experimental stroke in rats. Brain Res. 1315, 128-136. doi: 10.1016/j.brainres.2009.12.001

[31] Lemarchant, S., Dunghana, H., Pomeshchik, Y., Leinonen, H., Kolosowska, N., Korhonen, P., et al. (2016). Anti-inflammatory effects of ADAMTS-4 in a mouse model of ischemic stroke. Glia 64, 1492-1507. doi: 10.1002/glia.23017

[32] Lim, J., Jeong, C., Jun, J., Kim, S., Ryu, C., Hou, Y., et al. (2011). Therapeutic effects of human umbilical cord blood-derived mesenchymal stem cells after intrathecal administration by lumbar puncture in a rat model of cerebral ischemia. Stem Cell Res. Ther. 2:38. doi: 10.1186/scrt79

[33] Lim, S. T., Airavaara, M., and Harvey, B. K. (2010). Viral vectors for neurotrophic factor delivery: a gene therapy approach for neurodegenerative diseases of the CNS. Pharmacol. Res. 61, 14-26. doi: 10.1016/j.phrs.2009.10.002

[34] Liu, H., Honmou, O., Harada, K., Nakamura, K., Houkin, K., Hamada, H., et al. (2006). Neuroprotection by PlGF gene-modified human mesenchymal stem cells after cerebral ischaemia. Brain 129, 2734-2745. doi: 10.1093/brain/awl207

[35] Mohamad, O., Drury-Stewart, D., Song, M., Faulkner, B., Chen, D., Yu, S. P., et al. (2013). Vector-free and transgene-free human iPS cells differentiate into functional neurons and enhance functional recovery after ischemic stroke in mice. PLOS ONE 8:e64160. doi: 10.1371/journal.pone.0064160

[36] Niu, J., Li, C., Wu, H., Feng, X., Su, Q., Li, S., et al. (2015). Propidium iodide (PI) stains Nissl bodies and may serve as a quick marker for total neuronal cell count. Acta Histochem. 117, 182-187. doi: 10.1016/j.acthis.2014.12.001

[37] Nomura, T., Honmou, O., Harada, K., Houkin, K., Hamada, H., and Kocsis, J. D. (2005). I.V. infusion of brain-derived neurotrophic factor gene-modified human mesenchymal stem cells protects against injury in a cerebral ischemia model in adult rat. Neuroscience 136, 161-169. doi: 10.1016/j.neuroscience.2005.06.062

[38] Rafii, M. S., Baumann, T. L., Bakay, R. A. E., Ostrove, J. M., Siffert, J., Fleisher, A. S., et al. (2014). A phase1 study of stereotactic gene delivery of AAV2-NGF for Alzheimer's disease. Alzheimers Dement. 10, 571-581. doi: 10.1016/j.jalz.2013.09.004

[39] Rhim, T., and Lee, M. (2016). Targeted delivery of growth factors in ischemic stroke animal models. Expert Opin. Drug Deliv. 13, 709-723. doi: 10.1517/17425247.2016.1144588

[40] Rosenstein, J. M., Mani, N., Silverman, W. F., and Krum, J. M. (1998). Patterns of brain angiogenesis after vascular endothelial growth factor administration in vitro and in vivo. Proc. Natl. Acad. Sci. U.S.A. 95, 7086-7091. doi: 10.1073/pnas.95.12.7086

[41] Safiullov, Z. Z., Garanina, ÅÅ, Izmailov, A. A., Garifulin, R. R., Fedotova, V. Y., Salafutdinov, I. I., et al. (2015). Homing and survivability of genetically modified mononuclear umbilical cord blood cells after transplantation into transgenic G93A mice with amyotrophic lateral sclerosis. Genes Cells 10, 1-4

[42] Shcherbinin, D. N., Esmagambetov, I. B., Noskov, A. N., Selyaninov, Y. O., Tutykhina, I. L., Shmarov, M. M., et al. (2014). Protective immune response against Bacillus anthracis induced by intranasal introduction of a recombinant adenovirus expressing the protective antigen fused to the Fc-fragment of IgG2a. Acta Nat. 6, 76-84

[43] Sondell, M., Lundborg, G., and Kanje, M. (1999). Vascular endothelial growth factor has neurotrophic activity and stimulates axonal outgrowth, enhancing cell survival and Schwann cell proliferation in the peripheral nervous system. J. Neurosci. 19, 5731-5740

[44] Ström, J. O., Ingberg, E., Theodorsson, A., and Theodorsson, E. (2013). Method parameters' impact on mortality and variability in rat stroke experiments: a meta-analysis. BMC Neurosci. 14:41. doi: 10.1186/1471-2202-14-41

[45] Sun, T., and Ma, Q.-H. (2013). Repairing neural injuries using human umbilical cord blood. Mol. Neurobiol. 47, 938-945. doi: 10.1007/s12035-012-8388-0

[46] Taylor, R. A., and Sansing, L. H. (2013). Microglial responses after ischemic stroke and intracerebral hemorrhage. Clin. Dev. Immunol. 2013:746068. doi: 10.1155/2013/746068

[47] Wahl, A.-S., and Schwab, M. E. (2014). Finding an optimal rehabilitation paradigm after stroke: enhancing fiber growth and training of the brain at the right moment. Front. Hum. Neurosci. 8:381. doi: 10.3389/fnhum.2014.00381

[48] Wang, X.-L., Zhao, Y.-S., Hu, M.-Y., Sun, Y.-Q., Chen, Y.-X., and Bi, X.-H. (2013). Umbilical cord blood cells regulate endogenous neural stem cell proliferation via hedgehog signaling in hypoxic ischemic neonatal rats. Brain Res. 1518, 26-35. doi: 10.1016/j.brainres.2013.04.038

[49] Warren Olanow, C., Bartus, R. T., Baumann, T. L., Factor, S., Boulis, N., Stacy, M., et al. (2015). Gene delivery of neurturin to putamen and substantia nigra in Parkinson disease: a double-blind, randomized, controlled trial. Ann. Neurol. 78, 248-257. doi: 10.1002/ana.24436

[50] Yamashita, T., Deguchi, K., Nagotani, S., Kamiya, T., and Abe, K. (2009). Gene and Stem Cell Therapy in Ischemic Stroke. Cell Transplant. 18, 999-1002. doi: 10.3727/096368909X471233

[51] Yang, W.-Z., Zhang, Y., Wu, F., Min, W.-P., Minev, B., Zhang, M., et al. (2010). Safety evaluation of allogeneic umbilical cord blood mononuclear cell therapy for degenerative conditions. J. Transl. Med. 8:75. doi: 10.1186/1479-5876-8-75

[52] Yuan, T., Liao, W., Feng, N.-H., Lou, Y.-L., Niu, X., Zhang, A.-J., et al. (2013). Human induced pluripotent stem cell-derived neural stem cells survive, migrate, differentiate, and improve neurologic function in a rat model of middle cerebral artery occlusion. Stem Cell Res. Ther. 4:73. doi: 10.1186/scrt224

[53] Zhang, Z. G., Zhang, L., Jiang, Q., Zhang, R., Davies, K., Powers, C., et al. (2000). VEGF enhances angiogenesis and promotes blood-brain barrier leakage in the ischemic brain. J. Clin. Invest. 106, 829-838. doi: 10.1172/JCI9369

[54] Zhu, J., Liu, Q., Jiang, Y., Wu, L., Xu, G., and Liu, X. (2015). Enhanced angiogenesis promoted by human umbilical mesenchymal stem cell transplantation in stroked mouse is Notch1 signaling associated. Neuroscience 290, 288-299. doi: 10.1016/j.neuroscience.2015.01.038